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Hematological and serum biochemical aspects associated with a camel (*Camelus dromedarius*) naturally infected by *Trypanosoma evansi* with severe parasitemia in Semnan, Iran

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ABSTRACT

Objective: To determine the presence of *Trypanosoma evansi* (*T. evansi*) and the effect of trypanosomosis on hemato-biochemical profile of dromedary camels in Semnan, Iran, which has not been reported yet.

Methods: To perform this project, blood samples were collected by venipuncture into plain and EDTA-K2-containing vacutainer tubes from 21 dromedary camels (12 males and 9 females) aged 3-18 years, from 4 different regions of Semnan.

Results: Microscopic examination of stained thin blood smears revealed the presence of *T. evansi* in one of the samples. However, it should be noted that this sample showed a very high parasitemia (more than 5 trypomastigote were visible per microscopic field with MGG, 1 000×). This heavy parasitemia was associated with an 18-year-old female camel that showed symptoms of corneal opacity, intense emaciation and pale mucous membranes. Comparison of hematologyical and serum biochemical profiles between the camel infected by *T. evansi* and uninfected camels indicated anemia, leukocytosis, hyperproteinemia, hypoalbuminemia, hyperglobulinemia, reduction A/G ratio, increased α_1, β and globulins and decreased of α_2 globulins and increased the concentration of gamma-glutamyl transferase enzyme.

Conclusions: Results of the present study revealed that trypanosomosis was present in dromedary camels of Semnan, Iran (infection rate is 4.76%) and hemato-biochemical parameters were markedly affected by camel trypanosomosis.

1. Introduction

Trypanosomosis in camels is a protozoal disease caused by *Trypanosoma evansi* (*T. evansi*) which is transmitted by hematophagous flies including *Tabanus* and *Stomoxys*. Although trypanosomosis of camels occurs in both acute and chronic forms, it commonly occurs in the chronic form. Chronically infected camels show an intermittent fever, pale mucous membranes, corneal opacity, emaciation, thigh muscle atrophy, abortion and the loss of production[1]. Camel trypanosomiasis, also known as surra, is a disease causing morbidity up to 30.0% and mortality of around 3.0%[2]. Geographically, *T. evansi* has a wide distribution and affects many domestic animals, including camels, equines, donkeys, cattle, cats, dogs, buffaloes, small ruminants, carnivores and pigs. Surra disease in camels occurs in Asia, Africa, Central and South America and causes important economic losses[3-5]. According to available information, the prevalence of these diseases in camels in Iran is reported to be 10.0%[6]. Trypanosomosis diagnosis is made by identifying protozoa by direct microscopic smear preparations of blood. Trypanosomosis is responsible for significant changes in hematology and biochemistry parameters of infected racing camels[1].

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However, few studies have been done on hematological and biochemical alterations induced by natural trypanosomosis, but the influence of heavy infection with *Trypanosoma* on these parameters has not been investigated. Therefore, the purpose of this report was to evaluate the effects of heavy infection with *T. evansi* on hematological and biochemical profiles in a dromedary camel.

2. Materials and methods

For the determination of the presence of *T. evansi* infection in camels, 21 camels (12 males and 9 females) from 4 different herds located in Semnan county (36°0′ N, 54°0′ E) were randomly selected. The age of all camels used in this study ranged from 3 to 18 years old. Blood samples were collected by venipuncture into plain and EDTA-K2-containing vacutainer tubes from each camel.

The blood samples collected in ethylene diamine tetraacetic acid containing tubes were used to prepare thin blood smears for microscopic examination and to evaluate hematology profiles.

Thin blood smears prepared from each camel were stained by Giemsa and hematoxylin and eosin based on standard procedures and examined microscopically for the presence of trypanosome in circulation. The measured hematology parameters were red blood cell (RBC) counts, white blood cell (WBC) counts, packed cell volume (PCV), hemoglobin (Hb) concentrations, mean corpuscular volumes (MCVs), mean corpuscular hemoglobins (MCHs) and mean corpuscular hemoglobin concentrations (MCHCs). These parameters were estimated manually according to the method described by Tornquist[7].

To evaluate serum biochemical profiles, the blood samples in plain tubes were allowed to clot, and the serum after centrifugation at 3 000 r/min for 10 min was stored in single test tube at −20 °C until processing. The serum biochemical parameters measured included: total proteins, albumin, globulins, albumin to globulin ratios (A/G) and γ-glutamyl transferase (GGT) levels. Biochemical analyses were carried out using commercial kits (Pars Azmun, Tehran, Iran) according to manufacturer′s instructions. The colorimetric reactions were measured using a spectrophotometer (Biochrom WPA Biowave II). Serum protein fractions (α_1-, α_2-, β-, and γ-globulin) were separated using electrophoresis on cellulose acetate plate according to manufacturer′s instructions (Helena Biosciences, UK).

3. Results

Microscopical examination of the stained blood films determined the presence of *T. evansi* in one of the samples. However, it should be noted that this sample showed a very high parasitemia (more than 5 trypomastigote were visible per microscopic field at a magnification of 1 000×) (Figure 1). This heavy parasitemia was associated with an 18-year-old female camel that showed symptoms of corneal opacity, intense emaciation and pale mucous membranes.

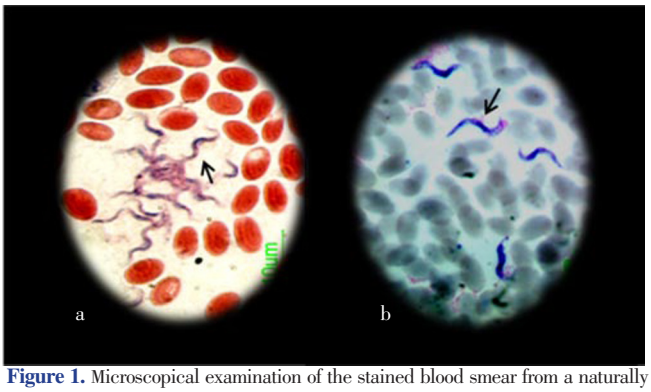

Figure 1. Microscopical examination of the stained blood smear from a naturally camel infected with *T. evansi* (arrow) (1 000×).
a: Hematoxylin and eosin-stained blood smear; b: Giemsa-stained blood smear.

Hematological parameters between the camel infected with *T. evansi* and uninfected camels are compared in Table 1. The parameters of PCV, Hb concentration, RBC counts, MCV and MCHC values were decreased, while WBC counts were increased in the *T. evansi*-positive sample (Table 1).

Table 1
Hematological parameters of *T. evansi*-negative and *T. evansi*-positive groups.

Parameters	*T. evansi* negative (*n*=20)	*T. evansi* positive (*n*=1)	SE	Change (%)
PCV (%)	28.33	23.00	4.47	−18.81
Hb (g/dL)	9.43	7.40	1.57	−21.52
RBCs ($\times10^6$/μL)	6.95	6.80	2.14	−2.16
MCV (fL)	44.58	33.82	15.81	−24.13
MCHC (g/dL)	33.33	32.17	2.35	−3.48
WBCs ($\times10^3$/μL)	8.55	16.00	2.74	46.56

Serum biochemical parameters between the camel infected with *T. evansi* and uninfected camels are compared and showed in Table 2. Parameters of albumin, A/G, α_2- and β-globulin were decreased, while total protein, globulins, α_1-, γ-globulin and GGT were increased in the *T. evansi*-positive sample (Table 2).

Table 2
Biochemical parameters of *T. evansi*-negative and *T. evansi*-positive groups.

Parameters	*T. evansi*-negative (*n*=20)	*T. evansi*-positive (*n*=1)	SE	Change (%)
Total protein (g/dL)	7.28	8.54	1.210	13.18
Albumin (g/dL)	3.81	3.27	0.710	−14.17
Globulins (g/dL)	3.46	5.27	1.230	52.31
α_1-Globulins (g/dL)	0.11	0.17	0.058	35.29
α_2-Globulins (g/dL)	0.24	0.17	0.100	−29.16
β-Globulins (g/dL)	1.11	0.93	0.210	−16.21
γ-Globulins (g/L)	1.67	2.35	0.730	40.71
A/G ratio	1.24	0.62	0.520	−50.00
GGT (IU/L)	22.47	43.93	12.890	95.50

4. Discussion

One of the most important diseases among camels that causes serious economic losses in America, Africa and Asia, including Iran, is surra[8]. This study is the first report of trypanosomosis in camels in Semnan, Iran.

In the present study, decreased hematocrite level, Hb concentration, total erythrocyte counts, MCV and MCHC values and increased WBC counts in *T. evansi* infected camel compared to uninfected camels were observed. Anemia and leukocytosis are common features of trypanosomosis in camels[9]. Anemia appears to be predominantly caused by hemolytic associated with decrease life span of erythrocytes and extensive erythrophagocytosis[10]. Mechanisms involved

in the development of anemia include: hemolysis, free fatty acids, immunologic mechanisms, hemodilution, coagulation disorders, depression of erythrogenesis and release of trypanosomal sialidase[10-13]. The most important oxidative enzyme during trypanosomosis is sialidase. Sialic acid in erythrocyte surface membrane is hydrolysed by sialidase[14].

Leukocytosis is the result of increased activity of the mononuclear phagocytic system during trypanosomiasis. These results are similar to the findings of Chaudhary and Iqbal, and Padmaja[1,15].

To determine the functional status of various organs investigated serum biochemical profile is a good indicator. Total protein, albumin, globulins, A/G ratio, α_1, α_2, β and γ-globulin and GGT enzyme were studied in one smear positive sample and 20 smear negative samples. Hyperproteinemia and hyperglobulinemia in the *T. evansi* infected camel compared with uninfected camels may be related to the presence of trypanosomes in the blood stream, which stimulates the immune system to secrete immunoglobulins[16]. In response to the animal trypanosomosis, especially the chronic form of infection, IgM levels often increase[16,17]. Therefore, increased immunoglobulin levels can lead to increased concentrations of serum total protein in our study.

Severe degenerative changes in the liver, which was confirmed cytologically by the presence of hepatic necrosis, may be hypoalbuminemia[1,18]. With hyperglobulinemia in trypanosomosis, hypoalbuminemia may be a compensatory mechanism to maintain osmolarity[3].

The decrease in the A/G ratio is related to the presence of hypoalbuminemia and hyperglobulinemia[1,18]. The increase in γ-globulin can be caused by increased immunoglobulin concentration, as was previously reported by Khosravi *et al.*, and Taylor and Authié[4,16]. Serum GGT activity is largely derived from the hepatobiliary system. Increasing serum GGT activity observed in the present study may be a result of the fatty degeneration of the liver cells and subsequent liver necrosis[19].

Results of the present study revealed that: first, trypanosomosis is present in dromedary camels of Semnan County, Iran (infection rate is 4.76%) and this is the first study of this protozoa infection in the region of Iran. Secondly, hematological and biochemical parameters are markedly affected by camel trypanosomosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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